Effect of Microhabitat, Light and Temperature on Seed Germination

6.1 Introduction

Seedling emergence and survival in plants differ greatly in their habitat preferences. Young seedlings on the forest floor are susceptible to many threats; recruitment of a species is possible in microhabitats having fewer threats. The characteristics of the microsite occupied by a seed may strongly influence its probability of germination and subsequent survival; species that live in highly specific habitats often produce seeds with highly specialized adaptations (Navarro and Guitian, 2003). Highly specific microhabitats may affect seed germination and subsequently seedling survival. Specific, though, congenial micro-habitats may offer high chances of establishing gregarious and massive populations even for a rare and endangered species (Badola and Pradhan, 2010).

Temperature regulates the seed germination in three ways (Bewley and Black, 1994), (i) by determining the capacity and percentage of germination; (ii) by eliminating primary and/or secondary dormancy; and (iii) by inducing secondary dormancy. The optimal germination temperature for most seeds which do not acquire dormancy is 25^0C to 30^0C. However, temperature range for germination depends largely on, (1) period of the year in which plants complete their life cycle, and (2) geographic origin of the species (Besnier, 1989).

In addition, light is also important for seed germination. In the case of light sensitive species, the seed will not germinate if it remains covered by litter or soil. Many species respond to the environment with optimal growth and development according to the light they receive (Maloof *et al.*, 2000). Some seeds germinate similarly in light and darkness (Baskin and Baskin, 2001), while others do it more readily either under light (Colbach *et al.*, 2002) or darkness conditions (Thanos *et al.*, 1989). Also, light requirements for germination can vary with temperature. It has been demonstrated

that some species need a constant temperature and light to germinate and others can germinate either under light or darkness conditions but need temperature fluctuations (Felippe, 1978).

Swertia chirayita Roxb. ex Fleming H. Kharst (Gentianaceae) is a perennial herb distributed in temperate Himalaya (1200m – 3000m asl) from Kashmir to Bhutan and in Khasia hills of Meghlaya, India (Chanda, 1976). In general, 1800-2300m altitude is a suitable range preferred by the species in Sikkim Himalaya (personal observation). Usually, species attains two forms in its 3 year life cycle; for the first two year, it remains in rosette form and during the third year, it gives out shoot, flower and fruit and becomes ready for harvest by the end of the season. However, it has been observed rarely that the plant completing its life cycle in two years which can be perceived as an abnormal behaviour (personal observation). Wind plays vital role in seed dispersal; the species is self pollinated (Chakroborty *et al.*, 2009), however, different pollinators have been observed during flowering season (personal observation).

Ethnomedicinal use of *S. chirayita* is known to exist prior to subsistence of modern health care system, for having numerous medicinal properties (Nadkarni, 1976; Biswas and Chopra, 1982; Kirtikar and Basu, 1984; Wawrosch *et al.*, 2005; Pradhan and Badola, 2008). It is a source of important chemical compounds such as, amarogentin (chirantin), gentianine, swerchirin and swertiamarin (Singh, 2008). Whole plant is known to possess anti-inflammatory, anti-platelet, anti-cancerous, anti-fungal and anti-malarial (Brahmachari *et al.*, 2004), anti-viral properties (Verma *et al.*, 2008). The raw material demand of *S. chirayita* is huge in both domestic and international market (Bhattarai ˙and Acharaya, 1997; Badola and Pal, 2002, 2003; Badola and Aitken, 2003, Dutta, 2004, Olsen, 2005). .

Due to indiscriminate extraction and habitat loss for several reasons, in many parts of Himalaya, *Swertia chirayita* has become critically endangered (Ved *et al.*, 2003a; IUCN, 2008) and is under the threats of extinction, and is a top priority species for its immediate conservation through *ex-situ* mechanism (Badola and Pal, 2002).

Conventional propagation of the species is possible only through seeds, and available literature reveals low percent germination in *S. chirayita* (Raina *et al.*, 1994; Basnet, 2001). There have been no previous studies available on the influence of microsites, as source, with light and temperatures, on seed germination in *S. chirayita*. Understanding germination behaviour of seeds from different microhabitats would help field managers and scientists to implement conservation strategies (Cervera *et al.*, 2007), particularly in case of species recovery programme of the species. The present study has been undertaken with the objectives to address these questions: (i) do seed germination variability exists amongst microhabitats in *Swertia chirayita*? (ii) what is the optimal temperature requirement for its seed germination?; and (iii) are the seeds light sensitive?

6.2 Materials and Methods

6.2.1 Seed germination test

The study was carried out with the seeds of *Swertia chirayita* harvested during December from 6 different microhabitats, viz., tree base, stump base, boulder, under canopy, shrubberies and grassy slope, from the forests of East Sikkim, India, (ca. 2000m asl). Mature fruits/capsules were collected and brought to the laboratory. The seeds were separated from fruits. After removing available impurities, the seeds were room dried for 10 days, and soaked for 24 hrs in double distilled water separately for each microhabitat. The soaked seeds were surface sterilized by immersing in Sodium hypochlorite (available chlorine 4.0%, E. Merck Company, India) solution for 5 sec. and washed thoroughly with double distilled water before placing in petri plates (90mm) lined with single layer Whatmann no. 1 filter paper.

Three sets (set A, B and C) in alternating light and dark photoperiod (14/10 light/dark), and three sets (set A1, B1 and C1) in continuous darkness (petri plates wrapped with aluminum foil) for each microhabitat were placed in a germination chamber at constant temperature of 20^0C, 25^0C and 30^0C, respectively. Each set of experiment had 3 replicates of 30 seeds each. Double distilled water was added whenever needed. The seeds were observed daily, and considered germinated upon radical emergence. On germination, the seeds were counted and removed. The

experiment was completed in 45 days. Set A1, B1 and C1, showing no germination or very poor germination in 45 days in continuous darkness, were then transferred to 14/10 hrs light/dark photoperiod individually at three continuous temperature regimes for another 45 days to determine the possibility of seed germination recovery in *S. chirayita*.

6.2.2 Statistical analysis

Statistical analysis was performed using SPSS 10.0 software for Windows. The data were submitted to Two-way ANOVA, to demonstrate the significance of main factors (seed source and temperatures) and their interaction in affecting the percent seed germination, germination rate, germination recovery percent ·and germination recovery rate. A Bonferroni test was used to determine if differences among means are significant ($p < 0.05$). Mean germination rate (r) was calculated according to Labouriau (1972) as, $r = 1/\sum(xd)/\sum t$ where 'x' is number of newly emerged seedlings in each day d, and 't' is total number of seedling emerged at the end of the test. Percent germination recovery (GR) was determined from the expression, % GR = $[(b/n_i) - (a/N)] \times 100$ where "a" is number of seeds germinated in continuous darkness from the actual total seeds "N", "b" is number of seeds germinated after transferring the seeds to 14/10 hours light/dark photoperiod from the remaining seeds "n_i".

6.3 Results

Table 13 shows the effect of microclimatic conditions and the temperature on percent seed germination, germination rate, germination recovery percent and germination recovery rate. Over 70% seed germination was recorded in alternating light and dark photoperiod from all the seed sources and temperature conditions except grassy slope (68.89%; 20^0C) [Figure 15A]. Seeds sourced from tree base, stump base and canopy resulted in highest of 82.22% germination at 20^0C which was significantly higher than grassy slope (68.89$\pm$5.09; $p < 0.05$) and non-significantly higher than shrubberies (71.11$\pm$1.92); similarly, at 25^0C, stump base (97.78$\pm$3.85%) recorded highest germination value followed by boulder (94.44$\pm$5.09%), tree base (92.22$\pm$5.09%) and canopy (91.11$\pm$1.92%). These values were significantly higher than the values recorded for shrubberies (77.78$\pm$3.85%; $p < 0.05$). Further, the highest value for stump

base was significantly higher compared to grassy slope (84.44+1.93%; p<0.05). At 30^0C, the maximum seed germination recorded for boulder (87.78+1.92%) and canopy (86.67+3.33%) was significantly higher (p<0.05) than the lowest recorded for shrubberies (73.33+3.33%) and grassy slope (75.56+1.93%). Seeds sourced from tree base and stump base recorded 83.33+5.77% and 83.33+3.33% seed germination. When compared amongst the different temperature conditions, seeds incubated at 25^0C resulted in highest seed germination followed by 30^0C and 20^0C (Figure 15A). In the case of seeds collected from stump base, boulder and grassy slope, significantly high (p<0.05) seed germination was observed at 25^0C compared to 30^0C incubation. Also, for seeds from stump base, the seed germination was significantly high (p<0.05) at 25^0C compared to 20^0C. Contrarily, in continuous darkness, the seeds either very poorly germinated or did not germinate under any of the three temperature regime (Figure 15B).

After 45 days, the non-germinated seeds when transferred from dark condition to 14/10 hours light/dark photoperiod significantly recovered in germination for all the microhabitat conditions and temperature regimes (Figure 15C). However, variation in germination recovery percent was observed. For example, at 20^0C, significantly (p<0.05) highest seed germination recovery percent was observed in seeds from under canopy (81.68+3.9) compared to other microhabitat condition (tree base: 65.09+5.92; stump base: 61.11+1.92; shrubberies: 41.11+6.94; grassy slope: 59.89+5.26) except boulders (77.78+5.09) in which the difference in seed germination recovery percent was non-significant. Similarly, the lowest seed germination recovery percent recorded for shrubberies was significantly low (p<0.05) compared to all other microhabitat conditions. The seed germination recovery percent was significantly high (p<0.05) in boulders compared to seeds from stump base and grassy slope. Similarly at 25^0C, highest seeds germination recovery percent recorded for seeds from under canopy (86.67+5.77) was significantly high (p<0.05) compared to seeds from tree base (70.0+6.67), stump base (65.56+1.93), shrubberies (42.22+5.09) and grassy slope (71.09+7.54), however, it was non-significant for seeds from boulders (83.98+3.94). The lowest value recorded for shrubberies was significantly low (p<0.05) compared to other microhabitat conditions. Seeds from stump base recorded significantly low

(p<0.05) seed germination recovery percent compared to boulders. At 30^0C, of all the microhabitats, seeds from shrubberies (45.56 ± 1.93) recorded significantly low seed germination recovery percent than other microhabitat conditions (tree base: 68.29 ± 1.39; stump base: 62.22 ± 8.39; boulder: 74.44 ± 1.93; canopy: 74.83 ± 3.98; grassy slope: 60.0 ± 5.77). Similarly, seeds from canopy and boulder recorded significantly high (p<0.05) germination recovery value than grassy slope. When compared amongst temperature, seeds incubated at 25^0C recorded non-significantly high germination recovery value in seeds from all the microhabitat conditions except shrubberies where the value was higher at 30^0C.

The mean germination rate (Figure 16A) and the germination recovery rate (after transferring seeds from complete darkness to light/dark photoperiod cycle) [Figure 16B] were significantly greater (p<0.05) at 25^0C than at 20^0C and 30^0C, comparatively; amongst microhabitats, the variation in germination rate (Figure 16A) was non-significant while germination recovery rate was significant (p<0.05) [Figure 16B]. At all the three temperature conditions, seed sourced from shrubberies recorded significantly (p<0.05) lower germination recovery rate.

6.4 Discussion

Both internal and external environmental factors strongly influence the seed germination in nature (Baskin and Baskin, 2001). The ecological conditions prevailing in a given habitat affect seed germination (Uysal *et al.*, 2006) as evident from our study in which the variability in seed germination amongst the microhabitat conditions (sources) was observed. In nature, germination of seeds is often restricted to locations that meet specific environmental conditions (Vandelook *et al.*, 2008) and are often referred to as 'safe-sites' (Harper, 1977) or 'regeneration niches' (Grubb, 1977). Therefore, preservation of such safe-sites rather than restoration of habitat would be crucial for the conservation of the species. In present study, the seeds of *S. chirayita* when exposed to 14/10hours light/dark photoperiod under different temperature regime performed well, which coincide with day/night length in its natural environment. Plant species have a minimum temperature, an optimum temperature, and a maximum temperature requirement, above or below which their

seeds will not germinate (Cardoso, 1999; Yilmaz and Aksoy, 2007). In the present study, similar substantial effect on the percent seed germination was observed under different temperature conditions from 20^0C to 30^0C, and the seeds germination percent was higher at 25^0C than 20^0C and 30^0C. The effectiveness of alternating day and night temperature in stimulating seed germination compared to constant temperature (Yilmaz and Aksoy, 2007) was not applicable with *S. chirayita*, as high seed germination percent was achieved in all the seed sources at different temperature regime at constant day and night temperature.

The germinating seeds and seedlings are more susceptible to disturbance (Sharma *et al.*, 2009) under canopy as it provides refuge to many herbivore species, which results in trampling and foraging on seedlings of many important plant species due to which the survival rate of the seedlings may turn very low. As an adaptive mechanism, the plants grown under canopy produce seeds which tend to be more viable than the seeds produced by plants grown in open spaces. Such adaptation became evident in the present study, where high seed germination was recorded in the seeds sourced from tree base, stump base and under canopy than shrubberies and grassy slope. At the same time, there is a minimum variation in daily air and soil surface temperatures under the canopy of nurse plants than outside the canopy (Cervera *et al.*, 2006). Thus, the seedlings under canopy become protected from extreme temperatures by the nurse plants thereby increasing seedling survival and extending the species' distributional boundaries (Steenbergh and Lowe, 1977; Nobel, 1980) resulting in productive and healthy plants producing maximum viable seeds. Further, in the present study, germination percent value for seeds collected from boulders exceeded the value obtained for shrubberies and grassy slope. This might be due to the fact that in the bouldery areas, the chances of large number of seeds gathered in depressions during seed dispersal may result in very less seedling survival due to over-crowding. Therefore, the seeds produced from the survived plants tend to be more viable and productive. Thus, understanding microhabitat – seed germination relation *vis- a-vis* information on the micro-climate is vital in the field studies and for developing conservation models as well as helping effective management plans for endangered plant species (Cervera *et al.*, 2007) like *Swertia chirayita*.

The importance of light in seed germination depends upon the size of the seeds, and life form (annual or perennial) and the habitat of the species (Uysal *et al.*, 2006). In continuous darkness, the minute seeds of *S. chirayita* failed to germinate or showed very poor germination which might be due to induction of secondary dormancy. Yilmaz and Aksoy (2007) reported the greatest response of *Rumax scutatus* seeds to alternating light/dark regimes than total darkness. Similar light requirements for germination in many small seeded species have been reported (Pons, 1992). In *Marathrum schiedeanum* and *M. rubrum*, seeds were unable to germinate both in complete darkness and under far red light (Ortega *et al.*, 2009). *Achmea nudalis* failed to germinate in total darkness but *Streptocalyx floribundus* showed little germination, and red light highly improved seed germination in both *A. nudalis* and *S. floribundus* from 15^0C to 40^0C (Pinheiro and Borghetti, 2003). However, *Phragmites australis* seeds germinated well in both continuous darkness and light over most of the temperature regime tested (Ekstam *et al.*, 1999). According to Walck *et al.*, (2000), even a green safe light is sufficient to stimulate germination in positively photoblastic seeds. Very low germination recorded under continuous darkness in seeds of *S. chirayita* from some microhabitats might be due to exposure of seeds to white light for little duration during periodical checking for germination (Baskin and Baskin, 2001) which confirms the photobalstic nature of seeds of *S. chirayita*.

For all the microhabitats, seeds after incubation in continuous darkness for 45 days under 20^0C to 30^0C temperature regimes, when exposed to 14/10 hours light/dark photoperiod in the same temperatures regimes showed over 50% germination recovery, except in case of shrubberies. However, some variation in germination recovery was noticed amongst microhabitat sources, which could be due to differences in the temperature regime to which they are exposed (Gul and Weber, 1999). These responses are similar to those seeds which were directly incubated at 14/10 hours light/dark photoperiod at 20^0C to 30^0C temperatures, indicating the light sensitivity of *S. chirayita*. This ensures that the seeds will germinate successfully on or near the soil surface when other conditions including light are favourable for seedling emergence, and if the seeds are buried at soil depths below in darkness, they may remain the part of persistent soil seed bank (Qu *et al.*, 2008). In *S. chirayita*, of

three temperature regimes, higher seed germination and germination recovery value were obtained at 25^{0}C. Similar results were reported in *Rumax scutatus* (Yilmaz and Aksoy, 2007).

Our data showed that seed germination and germination recovery percents were higher in all temperature regimes from 20^{0}C to 30^{0}C, however, the best results obtained at 25^{0}C with high germination, germination recovery value and also high mean germination rate, suggesting it as the optimal temperature for *S. chirayita* achieving better performance. Habitat loss and degradation ameliorates the stressful condition (Cervera *et al.*, 2006) for the plant species threatening its existence including the targeted taxa. Significant variation in percent germination amongst microhabitats indicated that the maintenance of natural habitats is the best option to conserve the genetic diversity of these species; however, suitable propagation protocols would be a simultaneous requirement strengthening *ex-situ* conservation mechanism and entrepreneurship (cultivation for trade) programme (Badola and Pal, 2002; Badola and Singh, 2003; Badola, 2009). The human participation will, however, be vital in the recovery of endangered species (Maschinski, 2004) like *S. chirayita.*

6.5 Conclusion

Present study concludes with conformity that, in *S. chirayita*, (i) there is an influence of micro-habitats on seed germination; (ii) 25^{0}C is the optimal temperature requirement for better performance; and (iii) the seeds are light sensitive.

Table 13. Results of two way ANOVA showing effect of temperature and microhabitats and their interaction on various germination parameters in *Swertia chirayita*

Dependent variable	Independent variable		
	Temperature (T)	Microhabitat (M)	T x M
Germination (%)	45.50***	21.61***	1.46^{ns}
Germination rate	146.74***	1.06^{ns}	0.99^{ns}
Germination recovery (%)	7.27*	65.38***	1.27^{ns}
Germination recovery rate	5.90**	25.97***	0.78^{ns}

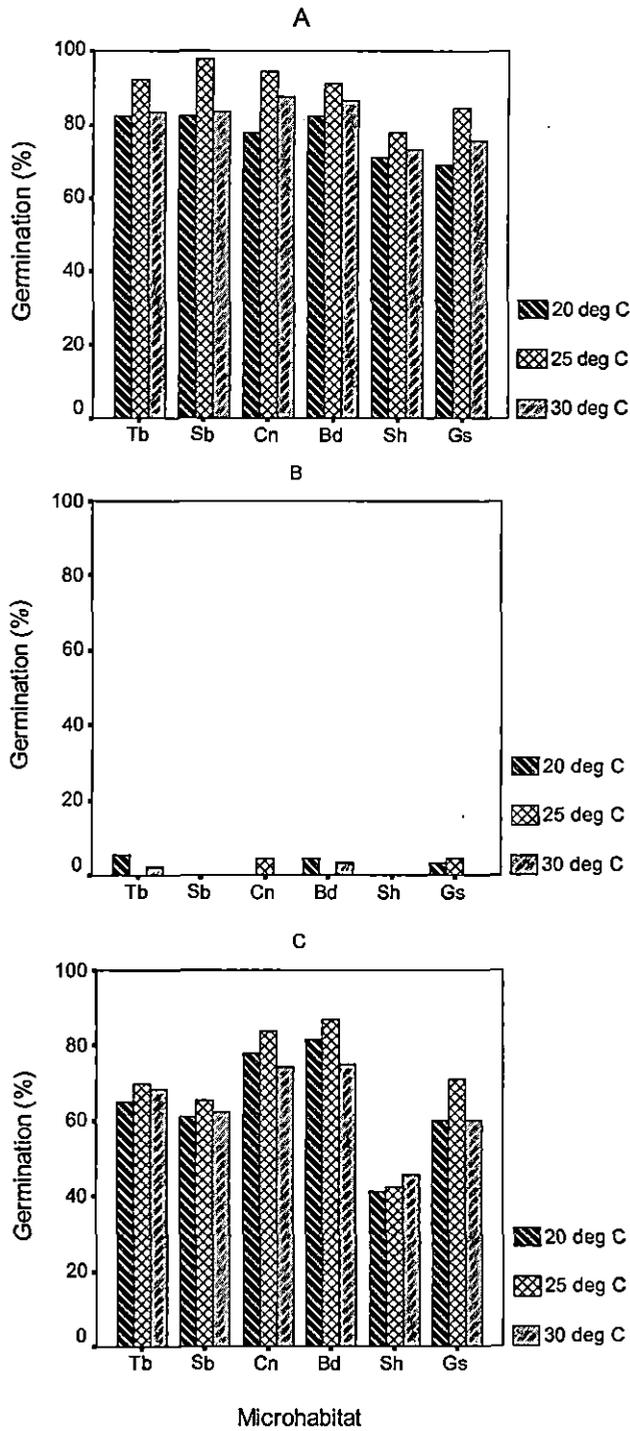* = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ns = not-significant

Figure 15. Seed germination response to various microhabitats and temperatures in *Swertia chirayita* (**A**: 14/10hrs. light/dark period, **B**: continuous darkness, **C**: transferred from continuous dark to 14/10hrs light/dark period after 45 days; Tb = tree base, Sb = stump base, Cn = canopy, Bd = boulder, Sh = shrubberies, Gs = grassy slope).
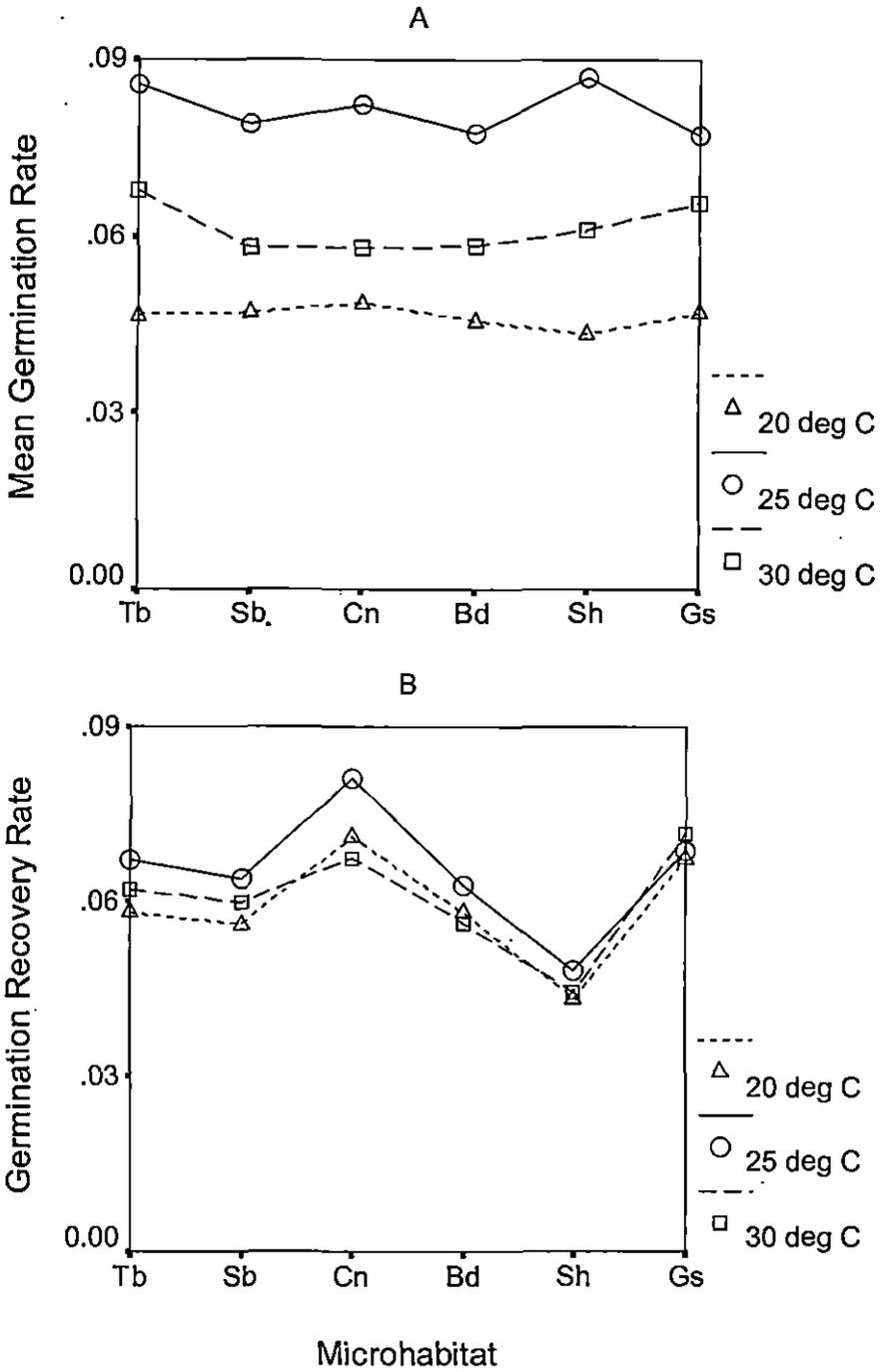
Figure 16. Microhabitat and temperature effect on germination rate and germination recovery rate in *Swertia chirayita* (**A**: Germination rate, **B**: Germination recovery rate; Tb = tree base, Sb = stump base, Cn = canopy, Bd = boulder, Sh = shrubberies, Gs = grassy slope).